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Molecular Analysis of Cellular Loci Disrupted by Papillomavirus 16 Integration in Cervical Cancer: Frequent Viral Integration in Topologically Destabilized and Transcriptionally Active Chromosomal Regions

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To discern the structural features of cellular loci that are disrupted by type 16 human papillomavirus (HPV-16) integration in cervical cancer, a polymerase chain reaction (PCR)-based strategy was employed for direct amplification and sequence analysis of four such cellular loci in cancer biopsy samples. One of the HPV-16-disrupted loci was found to be the microtubule-associated protein (MAP-2) gene and the other three loci were uncharacterized and were designated *PID-1* to *-3* (for papillomavirus integration-disrupted). The junctional sequences of the viral integration sites in the four loci analyzed are bracketed by long tracts of homogeneous purine or pyrimidine or alternating purine-pyrimidine which are known to destabilize the B-form conformation of the DNA structure. Using a panel of human/hamster hybrid cell DNAs and PCR analysis, the four loci were assigned to chromosomes 2 (MAP-2), 9 (*PID-1*), 1 (*PID-2*) and 8 (*PID-3*), respectively. These chromosomes carry numerous other previously determined viral integration and chromosomal fragile sites and the *myc* oncogenes. The *PID-1* locus was further found in Southern analysis to be rearranged and amplified in another cervical cancer biopsy and a cervical carcinoma cell line (CaSki). On Northern analysis, the *PID-1* and *-3* probes detected a 3.0- and a 3.6-kb transcript, respectively, in normal cervical cells and in cervical cancer cell lines. The findings suggest that HPV-16 genome integrates frequently into topologically destabilized and transcriptionally active chromosomal sites. It remains to be elucidated whether the *MAP-2* and the *PID* loci contribute to the pathogenesis of cervical cancer.

KEY WORDS: human papillomavirus, cervical cancer, viral integration, cellular locus

INTRODUCTION

The human papillomavirus (HPV) is a well-characterized DNA virus associated with anogenital lesions. On infection, the HPV sequence first exists as episomal molecules in high copy number [Matsukura et al., 1989; Cullen et al., 1991]. After a long period of incubation and as the infected lesions in the cervix progress to become low grade cervical intraepithelial neoplasia, the frequency of viral integration into one or more chromosomal sites drastically increases [Schneider-Maunoury et al., 1987]. About three-quarters of carcinoma cases contain HPV in the integrated form [Matsukura et al., 1989; Cullen et al., 1991]. More importantly, integrated HPV genomes are still transcriptionally active, expressing continually the oncogenic E6 and E7 genes [Schneider-Maunoury et al., 1987; Shirasawa et al., 1989]. Most cell lines derived from cervical cancer also contain integrated HPV-16 or -18, although cell lines with episomal HPV molecules have also been described [Choo et al., 1989].

The structure of HPV-16 integrants in a number of primary cervical cancer samples and cell lines has been characterized [Baker et al., 1987; El Awady et al., 1987; Choo et al., 1987, 1988; Chen et al., 1994]. For integration, the circular viral genome almost invariably linear-

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izes in the E1–E2 region resulting in the deletion of a part or the entire E1 and/or E2 sequences. Both the E1 and E2 proteins are essential components of the viral replication machinery [Chiang et al., 1992; Vecchio et al., 1992]. On the other hand, the E6 and E7 genes, and in most cases the long control region (LCR) which contains most of the regulatory signals needed for biological activities of the viral genome, are invariably retained on viral integration. The integrated HPV-16 sequences may be contiguous, or may contain internal deletions, duplications or sequence rearrangements [Choo et al., 1988]. The HPV-16 integrant may also be amplified together with the flanking cellular sequences [Lazo et al., 1989; Wagatsuma et al., 1990].

The mechanism of HPV integration is unclear but is thought to involve nonhomologous-type insertional recombination steps using short patches of sequence homology between the viral genome and the chromosomal integration site for transient sequence stabilization [Choo et al., 1990; Kahn et al., 1994]. No specific HPV-16 *integration sites* in the chromosome have been reported. However, chromosomal mapping studies have demonstrated a nonrandom association of HPV-16 integration regions with chromosomal fragile sites and oncogenes which suggests that viral integration may play a role in the activation of some oncogenes [Popescu et al., 1987; Cannizzaro et al., 1988; Lazo et al., 1989; Couturier et al., 1991]. HPV-16 integration also leads to deletion of some chromosomal sequences and inevitably results in chromosomal destabilization [Choo et al., 1990]. Briefly, the major effects of viral integration on the functions of the host genome are largely speculative and remain to be elucidated.

An important issue that needs to be addressed now is the structure of the chromosomal loci involved in viral integration, and whether disruption of some of these loci contributes to the pathogenesis of the uterine cervix. Although in some cases the integration sites have been found in repetitive sequences [El Awady et al., 1987; Wagatsuma et al., 1990], it is conceivable that the apparently random HPV integration may result in the disruption and inactivation of functional genes that are involved in the regulation of important cellular bioactivities. We now describe the analysis of four independent HPV-16 integration-disrupted cellular loci in different cervical cancer biopsies.

MATERIALS AND METHODS

Biopsy Samples and Cell Lines

The cervical cancer biopsies used in this study were squamous cell carcinoma samples. The mapping of the viral-cellular junctions of samples M15, V15 and V18 has been described previously [Chen et al., 1994]. The cervical cancer cell line CaSki was obtained from ATCC (CRL 1550).

Enzymatic Amplification and Selection of Viral-Cellular Junctional Sequences

For amplification of a previously mapped viral-cellular junction [Chen et al., 1994], 0.5 μg of the genomic DNA was used in a polymerase chain reaction (PCR) using the predetermined HPV-16 specific primer and a randomly chosen oligonucleotide primer [Parker et al., 1991]. For each junction, at least 30 different random primers were tested. The first PCR was carried out in a 50 μl volume in a thermal cycler (Thermal Cycler 480, Perkin-Elmer Cetus, Norwalk, CT) in 10 mM Tris.HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 0.1% Triton X-100, 200 μM of each of the dNTPs, 1.25 units Taq DNA polymerase (Promega, Madison, WI) and 5 pmole of each primer using the following PCR conditions: 94°C, 3 min for initial denaturation, and then 35 cycles at 94°C for 1 min, 40°C for 2 min, and 72°C for 2 min. The reaction was terminated after a further incubation at 72°C for 2 min. Following the first-round PCR reaction, 5 μl of the reaction mixture was used directly for a second-round PCR using a nested (internal) HPV-16 primer and the same set of random primers in a 50 μl volume in the same PCR buffer as above. The PCR conditions used at this time were: 94°C, 1 min for initial denaturation, and then 35 cycles at 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min. The reaction was terminated after an incubation at 72°C for 3 min. After the second-round PCR, 10 μl of the PCR products were electrophoresed in a 0.8% agarose gel, Southern-blotted and the membrane filter was used in a hybridization reaction using a HPV-16 probe. On autoradiography, HPV-16 positive bands were identified.

Thermal Cycle Sequencing

The HPV-16 bands identified after two rounds of PCR and a Southern hybridization step were excised from the agarose gel and the DNA was eluted. To obtain enough DNA for sequencing, the eluted DNA was subjected to another round of PCR using exactly the same conditions as for the second-round PCR described above. Such an amplification step normally produced only a single PCR product which was used directly in a thermal cycle sequencing reaction using a commercial sequencing kit (fmol™ Sequencing System, Promega, Madison, WI) in a thermal cycler. The internal HPV-16 primer was used as the sequencing primer using the following thermal conditions: 95°C for 2 min and then 25 cycles at 94°C for 1 min, 52°C for 1 min 20 sec and 70°C for 1 min 20 sec. The reaction products were analyzed in a 6% polyacrylamide gel as described previously [Choo et al., 1988].

Molecular Hybridization and Reverse Transcriptase (RT)-PCR Analyses

The preparation of genomic DNA and total RNA from biopsies and cultured cells and Southern and Northern hybridization analyses were carried out as described [Choo et al., 1988, 1990]. The *MAP-2* probe was derived from clone 47a [Lewis et al., 1986], a gift from Dr. N. Cowan (New York University Medical Center, NY). The *PID* probes were PCR-generated using primers shown in Table I. In the case of *PID-1*, a 290-bp *Ssp*I fragment was cleaved from the 510-bp PCR product to remove the apparently repetitive sequence. Detection of hybridization signals was achieved either by standard autoradiography using X-ray film, or by mounting on a Phosphor Screen™ followed by scanning in a Phosphor Imager™

TABLE I. Oligonucleotide Primers Used for the Generation of Probes for Southern Analysis and for the PCR-Based Chromosomal Assignment of the *PID* Loci

Locus (Sample)	Sequence (5′→3′)	PCR product Designation	PCR product Size (bp)
PID-1[a] (V15)	U: AAAGGCATTCTGGATAGCCC D: CTCTTCCTCTTCCCATAAGG	V15L	510
PID-2 (V18)	U: GCTAGGTATTGCTAAATTCC D: TGTTCTGGGCAATACAACAA	V18R	300
PID-3 (CC5a)	U: CATAATCGTTGTTGTGCTGG D: GAATGTGGCATTTCAGCAAT	CC5aR	520

[a]For the use as a *PID*-1 probe, a 290-bp fragment cleaved from the 510-bp PCR product using *Ssp*I restriction enzyme was used. For chromosomal assignment, the PID-1U primer was used in conjunction with another primer the sequence of which was 5′-AGACCTCAGCTAATTCCTAC-3′.

(Molecular Dymanics, Sunnyvale, CA). For RT-PCR analysis, a SuperScript™ RT-cDNA synthesis kit (BRL, MA) was used.

PCR-Based Chromosomal Assignment of Cellular Loci

For chromosomal assignment of the cellular loci, a PCR-based NIGSM human/rodent somatic cell hybrid mapping panel #2, version #2 [Dubois and Naylor, 1993], purchased from the Coriell Cell Repositories, Camden, New Jersey, was used. The primer sequences for mapping of the *PID* loci are listed in Table I. The PCR conditions used were as recommended by the supplier.

RESULTS

Derivation and Sequence Analysis of Viral-Cellular Junctions

As a first step towards the identification of HPV-16 integration-disrupted cellular sequences in cervical cancer, we established previously a PCR-based protocol for direct and rapid mapping of the viral-cellular junctions [Chen et al., 1994]. In this study, four cervical cancer biopsies (M15, V15, V18 and CC5a) with predetermined single-copy HPV-16 integrants were chosen for further studies. For a direct derivation of the flanking cellular sequences, we adopted a PCR approach based on the use of a single specific primer, the sequence of which was determined by the individual HPV-16 junction, in combination with a randomly chosen primer [Parker et al., 1991]. A two-round PCR protocol with increasing primer-annealing stringencies was applied in combination with a semi-nested PCR approach and Southern blot hybridization step using a HPV-16 probe to enrich and finally to identify the PCR products containing the desired HPV-16 junctional sequences. Representative gel profiles of the second-round PCR products obtained and the corresponding Southern blots are shown in Figure 1. In the case (sample M15) shown in Figure 1, five randomly chosen oligonucleotides were successful in amplifying the viral-cellular fragments to various intensities. The HPV-16-positive bands were eluted from the gel and were further amplified for use as templates in a thermal cycle sequencing reaction. Cellular sequences of 150 to 500 bp were routinely derived in this way.

In the four cervical cancer biopsy DNAs analyzed, seven viral-cellular junctions were successfully determined. For unknown reasons, we failed to obtain clear and specific HPV-16-positive hybridization bands for the upstream junction of sample V18 in numerous attempts. This junction was not analyzed further. The cellular sequences derived (partially shown in Fig. 2) were compared with the known sequences in the GenBank and EMBL nucleotide databases. One of the HPV-16-disrupted loci (sample M15) was found to be the microtubule-associated protein (*MAP-2*) gene. The viral genome integrated into the *MAP-2* gene in a transcriptionally reversed orientation relative to the HPV-16 sequence (Fig. 3). The identified cellular sequences of samples V15, V18 and CC5a were not in the databases and were tentatively designated *PID* (for papillomavirus integration disrupted)-*1*, *PID-2* and *PID-3* loci, respectively. Figure 3 depicts the seven integration junctions of the four loci in relation to the viral segments retained. For further analysis of the preinsertion sequences of *PID-1*, *-2* and *-3*, PCR-generated *PID* probes were used to screen a human genomic library. Sequences of the genomic clones in the vicinity of the preinsertion junctions were derived (Fig. 2). In sample M15, the *MAP-2* had suffered a 553-bp deletion from nucleotides 1201 to 1754 on viral integration.

Examination of the preinsertion sequences further shows that the junctions contain long homogeneous purine or pyrimidine stretches and alternating purine-pyrimidine tracts (Fig. 2). It is noteworthy that in sample M15, there was a 10-bp "filler sequence" (5′CTGTAATATG-3′) present at the upstream integration junction which was derived neither from the *MAP-2* nor the HPV-16 sequence at the integration junction (Fig. 3). However, a computer search revealed an identical sequence mapping at nucleotides 2353–2362 of the HPV-16 genome, a site at least 2 kb away from the determined viral integration point (Fig. 3). The mechanism of the generation of such a filler sequence is unclear [Roth et al., 1989].

Southern Analysis of the Viral Integration-Disrupted Loci

To characterize the structure of the four disrupted cellular loci, Southern analysis was carried out on the original biopsy DNAs. In the case of M15, a *MAP-2* cDNA

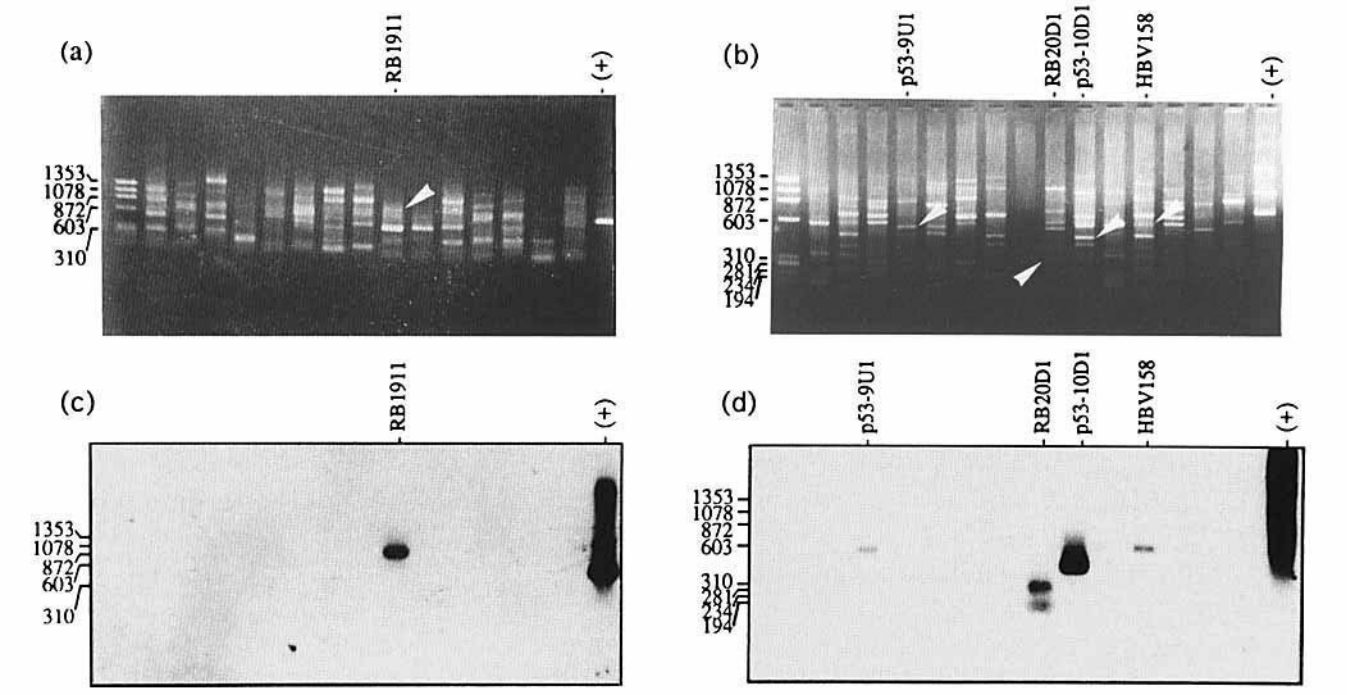

Fig. 1. Enzymatic amplification of the HPV-16 integration junctions in cervical cancer biopsy sample M15. Second-round PCR products generated using an internal HPV-16 primer for the upstream (**a**) and downstream (**b**) viral integration junctions (as depicted in Figs. 2 and 3) of the M15 DNA, respectively, are shown. The PCR products were analyzed in a 1.5% agarose gel. The designations of the successful random primers are indicated above the gel. The "+" lane contained HPV-16 plasmid DNA which was amplified using two HPV-16-specific primers to serve as PCR and hybridization controls. Arrowheads in a and b indicate the PCR bands that showed positive HPV-16 hybridization signals in the corresponding gels in **c** and **d**. Molecular weight markers (in bp) are shown on the left of the gels and Southern blots.

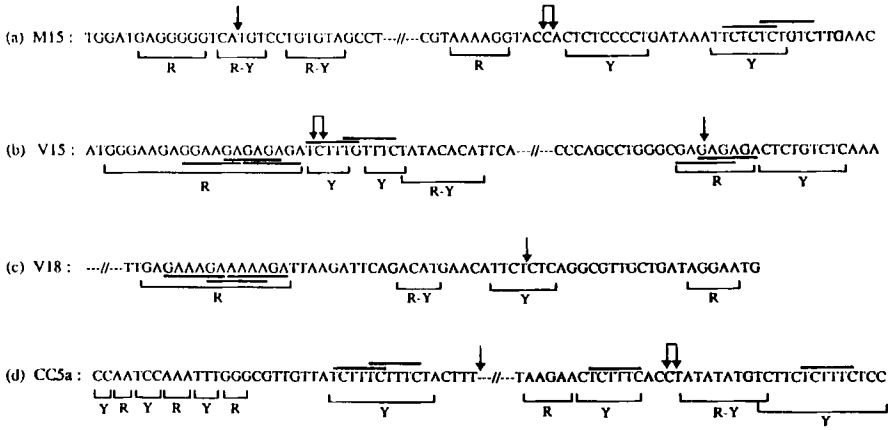

Fig. 2. Preinsertion sequences of the HPV-16-disrupted loci in the vicinity of the integration junctions. Vertical downward arrows indicate the positions of viral insertion. In cases where uncertainties occur due to the presence of a similar nucleotide in the viral and the cellular sequences at the junction, double arrows are shown. Orientation of each sequence is as depicted in Figure 3, arbitrarily normalized to the same HPV-16 transcriptional orientation. Homo-purine (R), homo-pyrimidine (Y), or alternating purine-pyrimidine tracts (R-Y) 5 nucleotides or longer are shown. In the upstream junction of the *PID-3* locus in CC5a, purines or pyrimidines of 2 or 3 nucleotides long are also shown to demonstrate the presence of alternating purine-pyrimidine di- or trinucleotides. The TCTTTC and its reverse complement GAAAGA hexameric sequences, or their one-base variants, are indicated by overlines or underlines, respectively.

[Lewis et al., 1986] was used as the hybridization probe. For *PID-1* to *-3*, PCR-generated DNA fragments in the size range of 300 to 510 bp were used as probes (Table I, see also Fig. 3). In the case of the *PID-1* locus, in order to remove the suspected existence of some repetitive sequences (data not shown), a 290-bp *Ssp*I-fragment cleaved from the 510-bp PCR product was used. Rehybridization with a HPV-16 probe was also performed using the same membrane filters. Representative Southern blots of the loci are shown in Figure 4. In each instance, probe detected two different alleles, one of which corresponded to the normal allele (Fig. 4, lanes

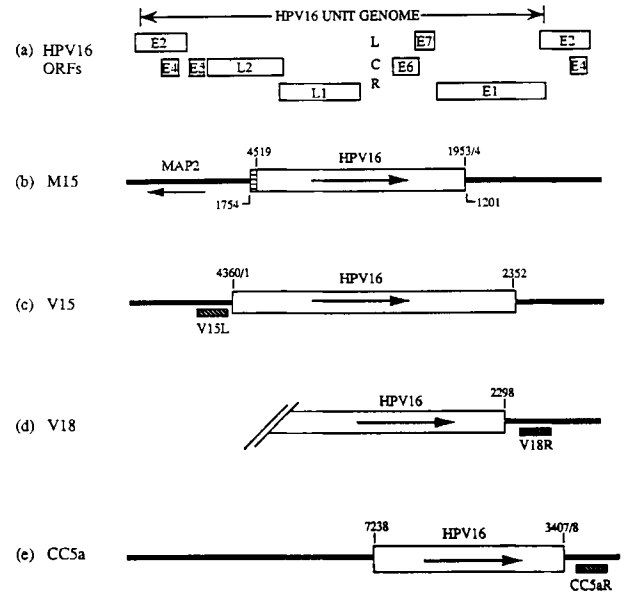

Fig. 3. The HPV-16 integration-disrupted loci. The direction of the loci is normalized to the same HPV-16 transcriptional orientation. **a:** Open reading frames of the HPV-16 genome. **b–e:** The HPV-16-disrupted loci. Open boxes indicate the retained HPV-16 sequences matching the relative locations of the viral ORFs as shown in a. Thick lines indicate the cellular sequences retained on viral integration. The nucleotide positions of the viral integration junction are shown at the top of the viral sequences. For cases where there was an overlap of a similar nucleotide at the junction, the 1-nucleotide uncertainties are also shown. In b, the narrow box showing horizontally hatched lines represents the 10-nucleotide "filler sequence" that matches a HPV-16 sequence mapping at nucleotide positions 2353–2362. Cross-hatched bars show the positions of the PCR-generated probes that were used in Southern and Northern analyses. Oligonucleotide primers were also designed from these regions of the cellular sequences for chromosomal assignments of the loci.

N) whereas the off-sized band was also HPV-16-positive, indicating that it was the HPV-16-disrupted allele.

To examine if the disruption of the four cellular loci by the HPV-16 integration is unique to the cases analyzed only, the *MAP-2* and the *PID* probes were further used in Southern blots of DNAs prepared from 16 cervical cancer biopsies and 8 cervical cancer cell lines. Only the *PID-1* probe detected novel bands in the Southern blots in one other cervical cancer biopsy (M8C) in addition to the original sample V15, and in a cervical cancer cell line, CaSki (Fig. 5). Biopsy sample M8C contained about 5 copies of HPV-16 and the CaSki cell line is known to contain 400–600 copies of integrated HPV-16 [Mincheva et al., 1987].

Northern Analysis of the Loci

To determine the transcriptional status of these cellular loci, Northern analysis was undertaken. No *MAP-2* transcripts were detected in RNA preparations of a normal cervix and a number of cervical cancer cell lines in Northern analysis. However, when the RNA preparations were subject to reverse transcriptase (RT)-coupled PCR analysis using primers specific for the *MAP-2* gene, a PCR product which hybridized to the *MAP-2* cDNA probe was observed (data not shown). The result indicates a very low level of *MAP-2* gene expression in cervical cells. The known lengthy size (9 kb) of the *MAP-2* transcript may have also contributed to the failure in the detection of the *MAP-2* transcript in Northern blots.

Of the three *PID* loci, the *PID-1* and *-3* probes detected transcripts of 3.0 and 3.6 kb, respectively, in a normal cervix biopsy and in a number of cervix cancer cell lines which included the HPV-16-positive CaSki cells (Fig. 6). RNAs from biopsy samples V15 and CC5a were not available for Northern analysis. The PCR-generated *PID-2* probe (300 bp) did not reveal any detectable transcripts. A much longer (6 kb) genomic fragment also failed to detect any *PID-2* transcripts when used as a probe in a similar Northern analysis (data not shown). The possibility that the sequences of the probes used fall within a large intron sequence of a gene cannot be ruled out.

Chromosomal Assignment of the *PID* Loci

For chromosomal assignment of the *PID* loci, oligonucleotide primers carrying locus-specific sequences (Table I) were used in a PCR analysis of a panel DNAs derived from human/rodent somatic cell hybrids each containing a single human chromosome [Dubois and Naylor, 1993]. *MAP-2*-specific primers were included as a control in the same experiment. The *MAP-2* gene was assigned to chromosome 2, in agreement with the known *MAP-2* chromosomal location of 2q34-35 [Neve et al., 1986]. The *PID-1*, *-2* and *-3* loci were clearly assigned to chromosomes 9, 1 and 8, respectively (data not shown).

DISCUSSION

A PCR-based approach is described for the analysis of cellular loci disrupted by HPV-16 integration. A salient feature of the preinsertion sequences at the viral integration sites is the presence of homogeneous purine or pyrimidine tracts, or alternating purine-pyrimidine sequences that were 5 nucleotides or longer (Fig. 2). The longest tract consisted of 19 nucleotides. In the hepatitis B virus integration sites in hepatocellular carcinoma and in the adenovirus integration regions in hamster cells, similar homopurine/homopyrimidine has been observed [Yaginuma et al., 1985; Ziemer et al., 1985; Tatzelt et al., 1992, 1993]. It has been estimated that a diploid human genome consists of 100,000 or more copies of polypurine/polypyrimidine repetitive elements [Schon et al., 1983]. Such asymetric nucleotide structures are known to destabilize the B-form conformation of the DNA molecule [Schon et al., 1983; Hoffman-Liedermann et al., 1986] which renders the sequences unfavorable for the binding of histone proteins [Arnott et al., 1983; Carbon, 1984]. This in turn results in a topologically destabilized chromatin conformation. It seems that topologically destabilized chromosomal regions are the preferred HPV-16 integration sites in cervical cancer.

It was observed that a hexameric sequence, TCTTTC, or its reverse complementary sequence, GAAAGA, or other one-base variants were consistently found in close proximity to both the upstream and downstream viral integration junctions in the four loci analyzed (Fig. 2).

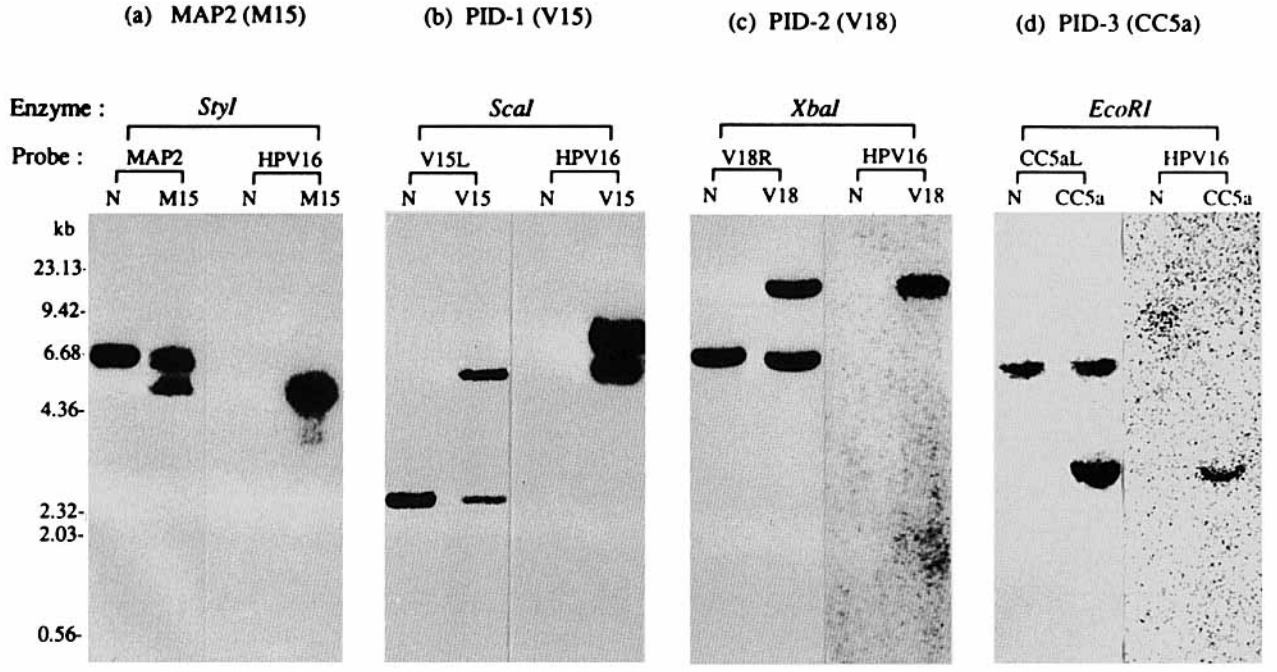

Fig. 4. Southern analysis of the HPV-16-disrupted loci. For sample M15, a *MAP-2* cDNA was used as a hybridization probe. For *PID-1* to *-3*, the probes were generated by PCR as shown in Figure 3. For each locus, the blot was sequentially hybridized with the cellular and the HPV-16 probes. Lanes marked "N" contained DNA prepared from the leukocyte cells of a normal individual.

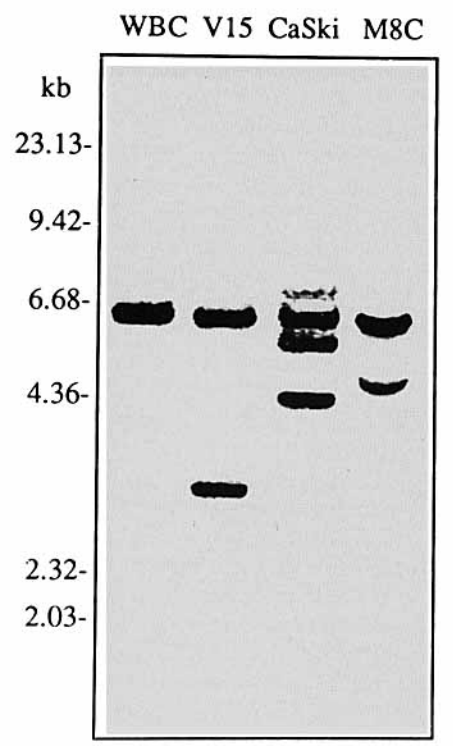

Fig. 5. Southern analysis of the *PID-1* locus in cervical carcinomas. The DNAs were digested with *Eco*RI for the analysis.

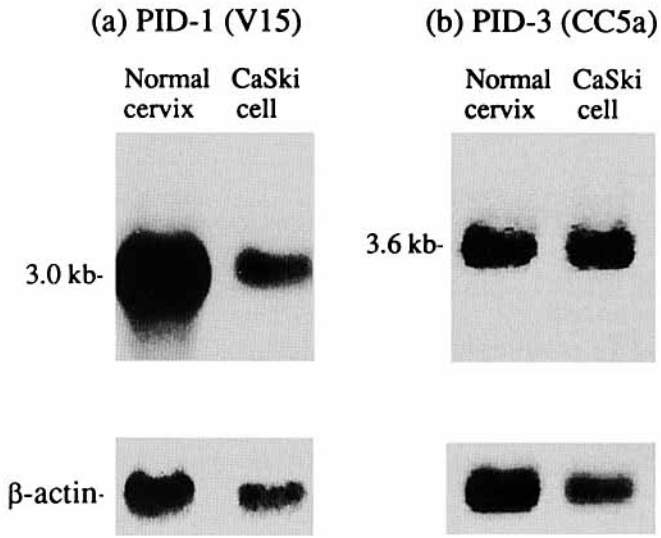

Fig. 6. Detection of *PID-1* **(a)** and *PID-3* **(b)** transcripts by Northern analysis. Total RNA preparations from a normal cervix and from the CaSki cell line were used. The *PID* probes were PCR-generated (see Fig. 3). After hybridizing with the *PID* probes, the membranes were stripped and were used in rehybridization with a human β-actin cDNA probe.

These hexameric sequences have also been found in the viral integration-disrupted cellular sequences reported previously [El Awady et al., 1987; Baker et al., 1987; Shirasawa et al., 1989; Choo et al., 1990]. The TCTTTC sequence bears close resemblance with a CTTTTT motif

of the hepatitis B virus genome and a CT(A/T)T(C/T)T motif of the parvovirus genome thought to be involved in the integration of these viruses in their respective hosts [Hino et al., 1989; Hogan and Faust, 1986]. The FLP recombinase cleavage site, CTATACTTTC, of the yeast 2 μm circles, also contains a CTTTC motif including the cleavage point C/T [Grono-Stajski and Sadowski, 1985]. The core sequence of the TCTTTC sequence, CTT, is an active topoisomerase I cleavage site which has previously been proposed to be involved in HPV integration [Choo et al., 1990; Kahn et al., 1994].

Three of the four loci determined were found to map in transcriptionally active regions of the chromosomes. Since only a small fraction of the human genome is transcribed and translated, the selection of such HPV-16 integration sites does not appear to be totally random but to be rather biased. The M15 locus is the *MAP-2* gene and the *PID-1* and *-3* loci, which have not been characterized before, also expressed a 3.0- or a 3.6-kb transcript in normal cervical cells, respectively. We have previously observed the disruption of the *jun-B* oncogene by a HPV-16 integrant in a cervical cell line [Choo et al., 1995]. Insertional recombination at transcriptionally active or DNase I hypersensitive sites has indeed been described for several sources of foreign DNA including proviral sequences of retroviruses and adenoviral sequences in different sources of target sites [Vajaya et al., 1986; Rohdewohld et al., 1987; Schulz et al., 1987; Tatzelt et al., 1993].

In the M15 integration site, the HPV-16 integration disrupted an exon of the *MAP-2* gene. MAPs are highly phosphorylated cytoskeleton proteins which serve to stabilize the microtubules against disassembly and to mediate interaction with other cellular structures. *MAP-2* gene is highly expressed in neuron cells [reviewed by Olmsted, 1986] but is apparently expressed only in very low levels in epithelial cells in general (this study). Some earlier studies have described detection of low levels of MAP-2 proteins in HeLa cells [Valdivia et al., 1982; Weatherbee et al., 1982]. It is not known if the *MAP-2* gene is transiently expressed at higher levels at other stages of tumorigenic progression.

The four loci analyzed in this study have been assigned to chromosomes 1, 2, 8 and 9. Although we have yet to map these loci regionally on the chromosomes, other studies have localized HPV integration sites on all these chromosomes [Popescu et al., 1987]. The assignment of the *PID-3* locus to chromosome 8 is particularly interesting since numerous HPV-16 or -18 integrants have already been mapped to chromosome 8 at 8q24 [Popescu et al., 1987, 1990; Durst et al., 1987; Mincheva et al., 1987; Cannizzaro et al., 1988; Lazo, 1988; Popescu and DiPaolo, 1989]. Nonrandom association of HPV-16 and -18 integration sites with the *myc* oncogenes on chromosome 8, and structural rearrangements, sequence amplification and elevated expression of the c-*myc* gene have been observed [Ocadiz et al., 1987; Durst et al., 1987; Choo et al., 1989; Couturier et al., 1991]. Interestingly, chromosomes 1 and 2 also carry members of the *myc* family, namely the L-*myc* and the N-*myc*, respectively. In HPV-18-transformed human keratinocyte cell lines, the viral genome has been reported to integrate in chromosome 1 at 1q12-q21 and 1p22-p31, in close proximity to known fragile sites [Smith et al., 1991]. Some early studies have shown that aberrations of chromosome 1 are frequently found in cervical cancer biopsies [Sreekantaiah et al., 1988; Atkin et al., 1993]. It remains to be determined if the *PID-2* and *-3* loci are associated with any of these known fragile sites or oncogenes or with the aberration-prone chromosomal regions. The cellular functions of the *PID-2* and *-3* gene products also need to be elucidated.

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